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Immunohistochemical analysis of polycyclic aromatic hydrocarbon-DNA adducts in breast tumor tissue

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Abstract

Environmental carcinogens may play a role in the etiology of breast cancer, but the extent of their contribution is not yet defined. The aims of this study were to determine whether polycyclic aromatic hydrocarbon (PAH)-DNA adducts could be detected in stored paraffin blocks of breast tumor tissue ($n = 147$) with an immunoperoxidase technique and whether they correlated with smoking history and/or mutant p53 protein expression. There was no significant difference in mean relative nuclear staining intensity in non-smokers (444 ± 90, $n = 75$), ever smokers (435 ± 91, $n = 72$), and current smokers (456 ± 98, $n = 35$). In either current or ever smokers, PAH-DNA adducts were non-significantly elevated in those with greater compared with lower exposure in relation to age at started smoking, years of smoking, cigarettes per day, and pack years. DNA damage levels were not elevated in tissues with compared with those without mutant p53 protein expression. These data demonstrate that immunohistochemical methods can be used to monitor DNA damage levels in archived breast tissues.

Keywords: Breast cancer; Immunohistochemistry; Polycyclic aromatic hydrocarbon-DNA adducts; Cigarette smoking

1. Introduction

Breast cancer is the second leading cause of cancer-related deaths among women in the United States. Of approximately 180 000 new cases of breast cancer diagnosed in 1997, about 44 000 women will die from the disease SEER [1]. Established risk factors for breast cancer include reproductive and menstrual characteristics, family history of breast cancer, post-menopausal obesity, and alcohol consumption [2]. However, taken together, these factors account for a little more than half of all breast cancer cases [3,4]; only 5–10% of cases result from inheritance of muta-

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tions BRCA1 and 2 and other high risk genes (e.g. ATM, p53) [5].

A number of chemicals to which there is widespread human exposure have been shown experimentally to cause mammary tumors in laboratory animals and to bind to DNA in breast epithelial cells [6]. These chemicals include polycyclic aromatic hydrocarbons (PAHs), which are ubiquitous pollutants formed as products of the incomplete combustion of fossil fuels. The main sources of PAH exposure are cigarette smoke, consumption of charbroiled and smoked foods, and air pollution. Benzo[*a*]pyrene (BP) is a representative PAH and can be metabolized to a highly reactive electrophile, which covalently binds to DNA [7]. This DNA damage can occur both early and late in the malignant process, thereby acting as an initiator and assisting in the progression of tumors [8]. Human adipose tissue can store BP [9] and the presence of adducts in DNA isolated from breast tissue in rodents is well documented [10].

Prior research has demonstrated an association between PAH-DNA adducts and a number of environmental exposures including workplace and air pollutants, cigarette smoke, and dietary intake (reviewed in [11,12]). In two recent studies using ^{32}P postlabeling among small samples of women, mean PAH-DNA adduct levels were found to be significantly higher in adjacent non-tumor breast tissue from cases than in normal tissues of breast reduction controls [13,14]. Epidemiological studies based on self-reported smoking histories have produced conflicting results with regard to the effect of smoking on breast cancer risk [15], with some investigators suggesting that it has both carcinogenic and anti-carcinogenic effects in the breast [16]. To date, breast cancer has not been strongly linked to occupational and environmental exposures to chemical carcinogens [6,17]. The uncertainty of the risk of smoking and other environmental and lifestyle factors in the etiology of breast cancer has increased the need for the use of biological markers such as DNA adducts for the assessment of the effect of environmental pollutants on breast cancer risk.

Highly specific antibodies to carcinogen-DNA adducts have been developed for measurement of human exposure to chemical carcinogens via immunologic methods [11,18]. In the present report, we used a quantitative immunoperoxidase method for the detection of PAH-DNA in paraffin blocks of breast tumor tissue. The assay used an antiserum generated against (±)-*r*-7,*t*-8-dihydroxy-*t*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) modified DNA which cross-reacts with other PAH diol epoxide adducts [19]. Therefore, adduct levels determined with this assay are referred to as PAH-DNA adducts. Tissues were from a subset of samples collected from the New Jersey component of the Women's Interview Study of Health (WISH), a multi-center, epidemiological study of breast cancer undertaken to determine whether long term oral contraceptive use, adolescent diet, lifetime use of alcohol, and other factors are associated with breast cancer risk among younger women [20–22]. In the present study, DNA damage was evaluated in relation to self-reported smoking status [22] and available data on mutant p53 protein expression in tumor tissue [21] to determine if smoking is correlated with increased DNA damage and whether mutant p53 expression is more frequent in those with elevated damage.

2. Materials and methods

This study used data on WISH [20] case participants who resided in five counties in central New Jersey. A woman was eligible as a case if she was newly diagnosed with in situ or invasive breast cancer between May 1990, and December 1992, was between the ages of 20 to 44 years, and was a resident of the five-county study area in New Jersey (Middlesex, Monmouth, Morris, Somerset, and Union). Breast cancer cases were ascertained using rapid reporting; personnel visited hospitals on a monthly basis to review pathology reports to identify eligible cases. A 70-min questionnaire was administered by trained individuals and included assessment of each respondent's family history of breast cancer, reproductive, menstrual, and contraceptive history, adolescent dietary intake, alcohol consumption, body size, physical activity, cigarette smoking, and other lifestyle factors. Interviews were completed with 509 cases (83.4%) of eligible cases.

The interview included details of usual cigarette smoking habits [22] including the age the respondent started and stopped smoking; total years smoked excluding years when the respondent did not smoke;

and intensity of smoking (number of cigarettes smoked per day, week, or year). An ever smoker was defined as having ever smoked 100 cigarettes or more, or having smoked one cigarette or more per day for 6 months or longer. A current smoker was defined as smoking at the reference date (date of the breast cancer diagnosis), or stopped within the 6 months prior to the reference date. A past smoker was defined as having stopped smoking at least 6 months or more before the reference date.

Paraffin-embedded tumor tissue blocks for immunohistochemistry were obtained from 39 of the 43 hospitals in the New Jersey area where the cases were diagnosed [21]. Blocks were successfully retrieved for 401 of the 509 interviewed cases and had been stored for 4–6 years before cutting of sections. For the present study, 155 cases were randomly selected from among those cases with available archived tissue. Data are available on 147 case subjects since some tissues were lost from the slides during staining.

Analysis was carried out on 5 μm paraffin sections, essentially as described previously [23]. After deparaffinizing, 10 mM citrate buffer, (pH 6.0) was added and the slides were boiled in a microwave oven twice for 5 min with a 1 min break. To enhance the sensitivity of the staining, phosphate-buffered saline (PBS) washed slides were treated with RNase A (100 μg/ml) at 37°C, washed with PBS and incubated with proteinase K (10 μg/ml at room temperature for 10 min), washed with PBS and 0.1 M HCl was added for 10 min at room temperature. The samples were neutralized with Tris base and washed again with PBS. Slides were incubated in 0.3% H_2O_2 in methyl alcohol at room temperature for 30 min to quench endogenous peroxidase activity. Non-specific binding was blocked by 10% normal goat serum. A 1:1000 dilution of polyclonal antiserum #1 [19] was incubated with the slides overnight at 4°C. After washing with PBS, peroxidase labeled anti-rabbit ABC and benzidine kits (Vector Laboratories, Burlingame, CA) were used. Staining was quantitated with a Cell Analysis System 200 microscope (Becton Dickinson, San Jose, CA) using the Cell Measurement Program software package. A total of 50 randomly selected cells from five fields, ten cells per field, were quantified and all data are expressed as the object average optical density multiplied by 1000.

Previous studies had demonstrated no significant daily variability in the immunoperoxidase staining of cells treated in culture with BPDE [23]. However, in the present study, the use of paraffin blocks necessitated the additional steps of deparaffinization and antigen retrieval by microwave heating. Since these procedures might introduce variability into the assay, paraffin-embedded lung tissues from an untreated mouse or one treated by i.p. injection with 1 mg of BP in corn oil for 16 h were stained with each batch of human samples. Repeat analysis of the BP-treated tissue indicated higher variability (1072 ± 234, $n = 9$, CV of 21%) in staining than prior results on cultured cells [23].

The specificity of staining was confirmed by omission of the primary antibody, preabsorption of the primary antiserum with BPDE-DNA (1 μg DNA/μl of serum for 30 min at room temperature followed by centrifugation) and pretreatment of tissues with DNase (100 μg/ml) for 1 h at 37°C before staining. Due to the limited number of slides from each individual, these controls were run on representative samples.

Cases were previously evaluated for evidence ofp53 protein expression by immunohistochemical staining as described, using p53 mouse monoclonal antibody clone D01 (Immunotech, Inc., Westbrook, ME) [21].

Data were analyzed using analysis of variance (ANOVA) procedures [24]. Tests of statistical difference in the mean and standard deviation of DNA damage levels were performed comparing non-smokers to ever smokers and non-smokers to current and former smokers. DNA damage levels were compared among different categories of smoking including age at starting smoking, years of smoking, cigarettes per day, and pack-years as well as by p53 status using ANOVA. These categories of smoking were analyzed for all smokers and then separately for current smokers only.

3. Results

A representative breast tumor tissue with high relative nuclear staining intensity (RSI) for PAH-DNA adducts is shown in Fig. 1A (RSI = 693), while a tissue with lower staining is shown in Fig. 1B

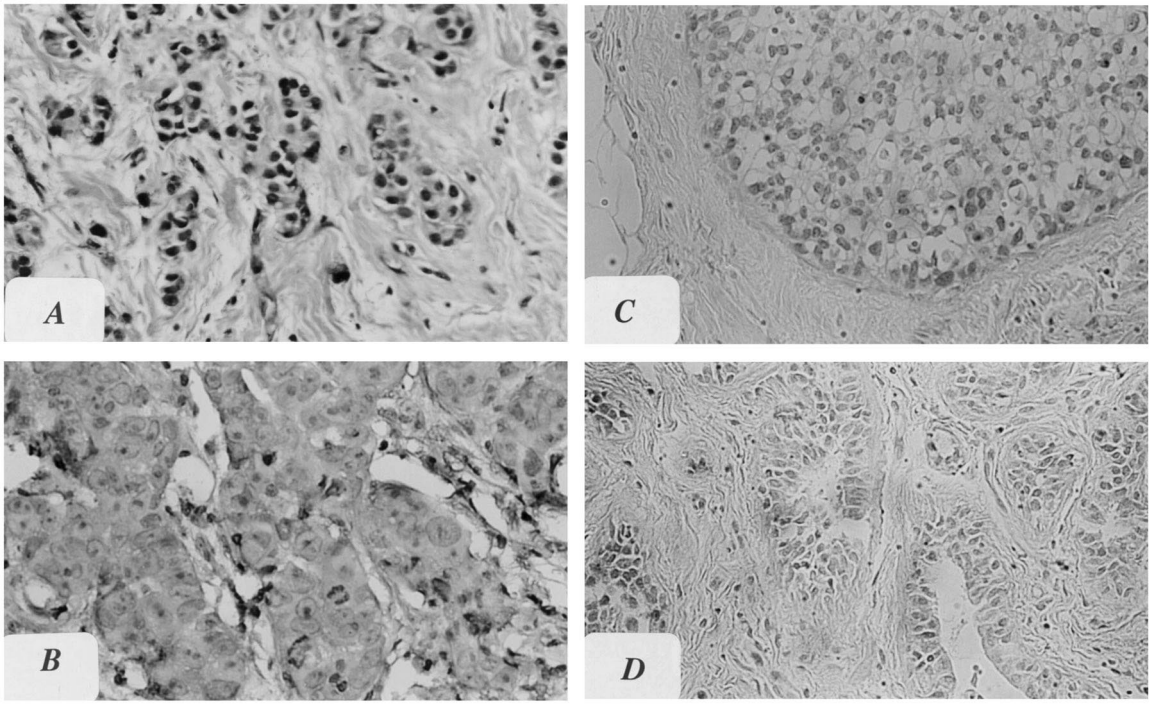

Fig. 1. Representative immunoperoxidase staining for PAH-DNA of paraffin-embedded breast tumor tissue. (A) Tissue section of a smoker positive for PAH-DNA, RSI = 693. (B) Tissue section of a smoker with background staining for PAH-DNA, RSI = 328. (C) Positive tissue section stained with primary antiserum recognizing PAH-DNA after preabsorption with BPDE-DNA, RSI = 202. (D) Positive tissue section stained for PAH-DNA after treatment with DNase, RSI = 136. (400× magnification).

(RSI = 328). To demonstrate the specificity of staining, an additional section of a positive tissue was stained with primary antiserum after preabsorption with BPDE-DNA (Fig. 1C). The RSI went from 638 to 202 with preabsorption of antiserum. After treatment of another sample with DNase (Fig. 1D) staining decreased from 619 without DNase treatment to 136 after treatment.

There was no significant difference in mean nuclear RSI among non-smokers (444 ± 104), ever smokers (435 ± 91), and current smokers (456 ± 98). In ever smokers, the various measures of smoking exposure, pack-years, age at starting smoking, years of smoking, and cigarettes per day, all showed an increased trend with higher exposures, although none of these results were statistically significant (Table 1). Since the stability of PAH-DNA adducts in breast tissue is unknown, we also analyzed the relationship between staining intensity and measures of smoking exposure in current smokers alone (Table 1). All measures of smoking exposure indicated higher levels of damage with higher exposure, but none of the results were significant.

Since data on p53 protein expression by immunohistochemical analysis were available on 142 subjects for whom PAH-DNA was measured [21], the correlation between these markers was also investigated. There was no difference in RSI for PAH-DNA in subjects positive for mutant p53 protein expression (425 ± 100, $n = 68$) compared with p53 protein negative subjects (448 ± 93, $n = 74$).

4. Discussion

Stored paraffin blocks are an excellent source of material for biomarker studies of breast cancer etiology. Here, we have demonstrated that PAH-DNA adducts can be measured in these tissues using an immunoperoxidase technique. Our hypothesis was

Table 1
Relationship between measures of smoking exposure and relative staining intensity for PAH-DNA adducts among combined current and former smokers and current smokers alone

	Ever smokers		Current smokers	
	N	Mean ± SD	N	Mean ± SD
Packyears				
0–<20	53	426 ± 90	20	446 ± 100
20+	19	459 ± 90	15	468 ± 97
Age started smoking				
18+ years	34	414 ± 87	19	424 ± 93
<18 years	38	453 + 91	16	492 ± 92
Years of smoking				
0–<20 years	54	425 ± 87	19	448 ± 95
20+ years	18	464 ± 98	16	465 ± 104
Cigarettes per day				
0–<20 cigs./day	41	424 ± 88	17	444 ± 95
20+ cigs./day	31	448 ± 94	18	466 ± 102

that smoking, in particular, current smoking, would increase PAH-DNA adducts in breast tissue. While there was a trend for increased levels of PAH-DNA in those with longer duration or higher consumption of cigarettes, the results were not statistically significant. Preliminary results were reported in abstract form by Li et al. [25] on PAH-DNA adducts in normal breast tissue of cases using our mono clonal antibody, 5D11. While the percentage of positively stained samples was reported, no information on smoking status was given. In agreement with the present results, two recent small studies using 32p postlabeling also found no relationship between smoking and PAH-DNA adduct levels in breast tissue [13,14]. In contrast, our previous study to detect PAH-DNA in exfoliated oral and bladder cells of smokers and nonsmokers, also using immunohistochemical methods, did find statistically significant differences related to exposure [26]. Thus, although the present study found no relationship between adducts and smoking, the method has observed a relationship in other tissues and suggests that the breast may not be as sensitive to the effects of smoking as other tissues.

This study has several limitations. The anti-PAH-DNA adduct antiserum used cross-reacts with several other structurally similar diol epoxide adducts with different affinities [19]. The human samples may contain varied amounts of these different adducts. Thus, it is not possible to determine absolute levels of DNA damage. The stability of the adducts paraffin sections is also uncertain. While several studies have investigated the stability of BP-DNA adducts in fresh or frozen tissues ([27] and references therein), to our knowledge there are no data on the stability of adducts in paraffin sections. Data on the positive control suggest that BP-DNA adducts are stable over the 6 month timeframe in which these studies were carried out.

Another limitation of the present study is that while a number of the paraffin blocks contained significant adjacent normal tissue, most sections contained only tumor tissue which may not be comparable to normal tissue in terms of carcinogen metabolism. The expression of some cytochrome P450s in human breast tissue has been found to be lower in tumor than in normal adjacent tissue from the same individuals [28]. There are few studies comparing DNA damage levels in tumor and adjacent normal tissue. Two previous studies in breast by ^{32}P postlabeling found no significant difference between tumor and adjacent non-tumor tissues [13,14]. Data on tissues other than breast are also limited. Our immunohistochemical studies on 4-aminobiphenyl-DNA (4-ABP-DNA) adducts found no significant difference in tumor compared with adjacent non-tumor liver tissues of hepatocellular cancer patients [29]. Nor did we find significant differences between tumor and non-tumor lung tissues for PAH-DNA [30]. Using ^{32}P postlabeling no significant differences were found in aromatic DNA adducts in tumor vs. non-tumor for larynx [31], but were found for colon [32]. Another study of lung with a small sample size ($n = 5$) found elevated adducts in normal tissue by postlabeling but not by ELISA [33]. The two different methods for adduct detection, used in these studies, have limitations in terms of types of adducts detected and accuracy of quantitative data. However, taken together, these results suggest that tumor tissue may be a reasonable source of material for assessing exposure to certain environmental exposures when normal tissue is not available.

The role of cigarette smoking in breast cancer etiology is controversial. Many epidemiological studies have found no relationship between smoking and breast cancer risk, while some studies have suggested that smoking at a younger age or for a long duration may increase risk [15]. In the young population of

women from whom the samples analyzed here were drawn, there was an apparent modest decrease in risk for breast cancer associated with current smoking [22]. One hypothesis suggests that risk may be reduced by smoking as a result of its anti-estrogenic effects [34]. Among premenopausal women, current smokers have lower luteal phase urinary levels of estradiol than subjects who never smoked [35,36].

P53 protein expression was previously analyzed by immunohistochemistry in tumor tissues that were analyzed here for DNA damage. Those findings support the hypothesis that p53 mutations in breast cancer are associated with current cigarette smoking [21]. A previous case-control study in Dutch women is in agreement also finding increased p53 mutations in current smokers [37]. The mutational pattern in the p53 gene in breast cancer cases partially resembles the pattern of lung cancer mutations, and may be related to environmental factors [38]. Twenty percent of the mutations in p53 in breast cancer cases are G → T transversions [39], consistent with DNA damage resulting from bulky carcinogens such as BP. However, we did not find a relationship between PAH-DNA adducts and expression of mutant p53 protein in the breast tumors. This is in contrast to our prior studies of hepatocellular cancer in which there was an increased odds ratio for mutant p53 protein in subjects with detectable aflatoxinB_1-DNA damage (OR = 2.9, 95% confidence interval 0.8–17.8) [40]. We also found a significant relationship in bladder cancer between 4-ABP-DNA and p53 staining [41]. In a study by others using gas chromatography/mass spectroscopy (GC/MS) to measure 4-ABP-DNA, adducts were not significantly elevated in those with p53 gene mutations compared with those without [42].

In summary, these results demonstrate that immunohistochemical methods can be used to obtain relative levels of DNA damage in stored paraffin blocks of tissue. They also confirm prior studies that environmental carcinogens such as PAH can reach breast tissue and damage DNA but that smoking does not significantly increase damage levels.

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References

[1] SEER, National Cancer Institute's Surveillance, Epidemiology, and End Results, Breast Cancer, January, 1997.
[2] J.L. Kelsey, L. Bernstein, Epidemiology and prevention of breast cancer, Ann. Rev. Publ. Health 17 (1996) 47–67.
[3] L.A. Brinton, J. Benichou, M.D. Gammon, D.R. Brogan, R. Coates, J.B. Schoenberg, Ethnicity and variation in breast cancer incidence, Int. J. Cancer 73 (1997) 349–355.
[4] P.M. Madigan, R.G. Ziegler, J. Benichou, C. Byrne, R.N. Hoover, Proportion of breast cancer cases in the United States explained by well-established risk factors, J. Natl. Cancer Inst. 87 (1995) 1681–1685.
[5] L.W. Ellisen, D.A. Haber, Hereditary breast cancer, Annu. Rev. Med. 49 (1998) 425–436.
[6] K. El-Bayoumy, Environmental carcinogens that may be involved in human breast cancer etiology, Crit. Rev. Toxicol. 5 (1992) 585–590.
[7] D.H. Phillips, Fifty years of benzo[a]pyrene, Nature 303 (1983) 468–472.
[8] S.H. Yuspa, M.C. Poirier, Chemical carcinogenesis: from animal models to molecular models in one decade, Adv. Cancer Res. 50 (1988) 25–70.
[9] H. Obana, S. Hon, T. Kashimoto, N. Kunita, Polycyclic aromatic hydrocarbons in human fat and liver, Bull. Environ. Contam. Toxicol. 27 (1981) 23–27.
[10] L.A. Seidman, C.J. Moore, M.N. Gould, 32P-postlabeling analysis of DNA adducts in human and rat mammary epithelial cells, Carcinogenesis 9 (1988) 1071–1077.
[11] R.M. Santella, Immunologic methods for detection of carcinogen-DNA damage in humans, Cancer Epidemiol. Biomarkers Prev. 8 (1999) 733–739.
[12] H.A.J. Schut, K.T. Shiverick, DNA adducts in humans as dosimeters of exposure to environmental, occupational, or dietary genotoxins, FASEB J. 6 (1992) 2942.
[13] D. Li, M. Wang, K. Dhingra, W.N. Hiffelman, Aromatic DNA adducts in adjacent tissues of breast cancer patients: clues to breast cancer etiology, Cancer Res. 56 (1996) 287–293.
[14] F.P. Perera, A. Estabrook, A. Hewer, K. Channing, A. Rundle, L.A. Mooney, R. Whyatt, D.H. Phillips, Carcinogen-DNA adducts in human breast tissue, Cancer Epidemiol. Biomarkers Prev. 4 (1995) 233–238.
[15] J.R. Palmer, L. Rosenberg, Cigarette smoking and the risk of breast cancer, Epidemiol. Rev. 15 (1993) 145–156.
[16] J.A. Baron, C. La Vecchia, F. Levi, The antiestrogenic effect of cigarette smoking in women, Am. J. Obstet. Gyn. 162 (1990) 502–514.
[17] D.L. Davis, M.J. Pongsiri, M. Wolff, Recent developments on the avoidable causes of breast cancer, Ann. N. Y. Acad. Sci. 837 (1997) 513–523.
[18] M.C. Poirier, Development of Immunoassays for the Detection of Carcinogen-DNA Adducts, in: J.D. Groopman, P.L. Skipper (Eds.), Molecular Dosimetry and Human Cancer: Analytical Epidemological and Social Considerations, CRC Press, Boca Raton, FL, 1991, pp. 211–230.
[19] J.L. Mumford, K. Williams, T.C. Wilcosky, R.B. Everson, T.L. Young, R.M. Santella, A sensitive color ELISA for

detecting polycyclic aromatic hydrocarbon-DNA adducts in human tissues, Mutat. Res. 359 (1996) 171–177.
[20] L.A. Brinton, J.R. Daling, J. Liff, J.B. Schoenberg, K.E. Malone, J.L. Stanford, R.J. Coates, M.D. Gammon, L. Hanson, R.N. Hoover, Oral contraceptives and breast cancer risk among younger women, J. Natl. Cancer Inst. 87 (1995) 827–835.
[21] M.D. Gammon, H. Hibshoosh, M.B. Terry, S. Bose, J.B. Schoenberg, L.A. Brinton, J.L. Bernstein, W.D. Thompson, Cigarette smoking and other risk factors in relation to p53 expression in breast cancer among young women, Cancer Epidemiol. Biomarkers Prev. 8 (1998) 255–263.
[22] M.D. Gammon, J.B. Schoenberg, S.L. Teitelbaum, L.A. Brinton, N. Potischman, C.A. Swanson, D.J. Brogan, R.J. Coates, K.E. Malone, J.L. Stanford, Cigarette smoking and breast cancer risk among young women, Cancer Causes Control 9 (1998) 583–590.
[23] T.M. Hsu, Y.J. Zhang, R.M. Santella, Immunoperoxidase quantitation of 4-aminobiphenyl-and polycyclic aromatic hydrocarbon-DNA adducts in exfoliated oral and urothelial cells of smokers and nonsmokers, Cancer Epidemiol. Biomarkers Prev. 6 (1997) 193–199.
[24] W.W. Daniel, Biostatistics: A Foundation for Analysis in Health Sciences, Wiley, New York, 1991.
[25] D. Li, W. Zhang, M. Wang, Y. Zhu, W.N. Hittleman, A. Sahin, Cellular distribution of aromatic DNA adducts in normal breast tissue of women with breast cancer, AACR Proc. 39 (1998) 491.
[26] Y.J. Zhang, T.M. Hsu, R.M. Santella, Immunoperoxidase detection of polycyclic aromatic hydrocarbon-DNA adducts in oral mucosa cells of smokers and nonsmokers, Cancer Epidemiol. Biomarkers Prev. 4 (1995) 133–138.
[27] B. Binkova, F. Hublek, R.J. Sram, Stability of benzo[a]pyrene DNA adducts in rat tissues during their long-term storage at −20°C or −80°C, Mutat. Res. 371 (1996) 229–235.
[28] Z. Huang, M.J. Fasco, H.L. Figge, K. Keyomarsi, L.S. Kaminsky, Expression of cytochromes P450 in human breast tissue and tumors, Drug Metab. Dispos. 24 (1996) 899–905.
[29] L.Y. Wang, C.J. Chen, Y.J. Zhang, W.Y. Tsai, P.H. Lee, M.A. Feitelson, C.S. Lee, R.M. Santella, 4-Aminobiphenyl-DNA damage in liver tissue of hepatocellular carcinoma patients and controls, Am. J. Epidemiol. 147 (1998) 315–323.
[30] D.L. Tang, R.M. Santella, M.A. Blackwood, T.L. Young, J. Mayer, W.Y. Tsai, F.P. Perera, molecular epidemiological case-control study of lung cancer, Cancer Epidemiol. Biomarkers Prev. 4 (1995) 341–346.
[31] K. Szyfter, K. Hemminki, W. Szyfier, Z. Szmeja, J. Banaszewski, K. Yang, Aromatic DNA adducts in lamyx biopsies and leukocytes, Carcinogenesis 15 (1994) 2195–2199.
[32] A.P. Leszkowicz, Y. Grosse, V. Carriere, P.H. Cugnenc, A. Berger, F. Camot, P. Beaune, I. de Waziers, High levels of DNA adducts in human colon are associated with colorectal cancer, Cancer Res. 55 (1995) 5611–5616.
[33] F.J. van Schooten, M.J.X. Hillebrand, F.E. van Leeuwen, N. van Zandwijk, H.M. Jansen, L. Den Engelse, E. Kriek, Polycyclic aromatic hydrocarbon-DNA adducts in white blood cells from lung cancer patients: no correlation with adduct levels in lung, Carcinogenesis 13 (1992) 987–993.
[34] K. Bennicke, C. Carsten, S. Sabroe, H.T. Serensen, Cigarette smoking and breast cancer, Br. Med. J. 310 (1995) 1431–1433.
[35] B. MacMahon, D. Trichopoulos, P. Cole, J. Brown, Cigarette smoking and urinary estrogens, N. Engl. J. Med. 307 (1982) 1062–1065.
[36] C. Westoff, G. Gentile, J. Lee, H. Zacur, D. Helbig, Predictors of ovarian steroid secretion in reproductive-age women, Am. J. Epidemiol. 144 (1996) 381–388.
[37] K. van der Kooy, M.A. Rookus, H.L. Peterse, F.E. van Lecuwen, p53 protein overexpression in relation to risk factors for breast cancer, Am. J. Epidemiol. 144 (1996) 924–933.
[38] R.E. Harris, E.A. Zang, J.I. Anderson, E.L. Wynder, Race and sex differences in lung cancer risk associated with cigarette smoking, Int. J. Epidemiol. 22 (1993) 592–599.
[39] P.J. Biggs, W. Warren, S. Venitt, M.R. Astratton, The value of mutational spectra in unravelling the etiology of cancer, Mutagenesis 8 (1993) 275–283.
[40] R.M. Lurm, Y.J. Zhang, L.Y. Wang, C.J. Chen, P.H. Lee, C.S. Lee, W.Y. Tsai, R.M. Santella, p53 Mutations, chronic hepatitis B virus infection, and aflatoxin exposure in hepatocellular carcinoma in Taiwan, Cancer Res. 57 (1997) 3471–3477.
[41] G. Curigliano, Y.J. Zhang, L.Y. Wang, G. Flamini, A. Alcini, C. Ratto, M. Giustacehini, B. Alcini, A. Cittadini, R.M. Santella, Immunohistochemical quantitation of 4-aminobiphenyl-DNA adducts and p53 nuclear overexpression in Ti bladder cancer of smokers and nonsmokers, Carcinogenesis 17 (1996) 101–106.
[42] T. Martone, L. Airoldi, C. Magagnotti, R. Coda, D. Randone, C. Malaveille, G. Avanzi, F. Merletti, A. Hautefeuille, P. Vineis, 4-Aminobiphenyl-DNA adducts and p53 mutations in bladder cancer, Int. J. Cancer 75 (1998) 512–516.